Research Article

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Histopathological Changes in Lungs and Tracheas of Broiler Chickens Infected With Infectious Bronchitis Virus

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Abstract

Infectious bronchitis is an acute extremely infectious respiratory illness caused by the avian gamma-corona virus. Infection with the infectious bronchitis virus predisposes the bird to secondary bacterial infection, worsening the situation. The infection causes severe morbidity and variable mortality in broilers, as well as a significant decrease in the layer production of eggs. This study was conducted to evaluate the pathological changes that occur in natural infection with the infectious bronchitis virus. Samples were collected from clinical cases submitted for necropsy at local veterinary clinics in Anbar province, Iraq. Tissues for molecular detection included tracheas, lungs, and kidneys. Samples confirmed infection by PCR used in the histopathological study. Histopathological sections from samples that tested positive showed variable lesions from one farm to another. In some cases, there was mild heterophilic infiltration and some times accompanied by deciliation and sometimes accompanied by subepithelial edema. In more severe cases there was congestion of blood vessels along heterophilic infiltration along with desquamation of the epithelial layers. The histopathological section in a lung of infected birds showed inflammatory cells infiltration consisting mainly of heterophils in the interstitial tissue. In some samples, there was extensive hemorrhage filling the atrial lumen and infiltration of eosinophils and heterophils along with interstitial hemorrhage. From this study, we concluded that viruses circulating in Iraq causes more pulmonary lesions when compared to lesions in the trachea.

Keywards: Infectious bronchitis virus, Chickens, Lung, Trachea

التغيرات النسجية في الجهاز التنفسي للدجاج اللاحم المصاب بالتهاب القصبات المعدي

الخلاصة

التهاب القصبات المعدي هو مرض تنفسي حاد شديد العدوى يسببه فيروس جاما-كورونا للطيور. العدوى بفيروس التهاب القصبات المعدي تجعل الطائر عرضة لعدوى بكتيرية لاحقة ، مما يؤدي إلى تفاقم الوضع. تسبب العدوى نسبة اصابة عالية ونفوق متفاوت في دجاج اللحم ، بالإضافة إلى انخفاض كبير في إنتاج الدجاج البياض. أجريت هذه الدراسة لتقييم التغيرات المرضية التي تحدث في العدوى الطبيعية بفيروس . تم جمع العينات من الحالات السريرية المقدمة للتشريح في العيادات البيطرية في محافظة الانبار في العراق. تضمنت أنسجة الكشف الجزيئي القصبة الهوائية والرئتين والكلى. وشملت عينات التشريح المرضي أنسجة من الرئتين والقصبة الهوائية. العينات التي تم اختبارها إيجابية للفايروس بالطريقة الجزيئية تم تقطيعها لفحص الأنسجة بينما تم التخلص من الحالات السالبة. اظهرت المقاطع النسيجية المرضية للطيور المصابة آفات متفاوتة من مزرعة الى اخرى. في بعض الحالات كان هناك تسلل خفيف لخلايا هتروفيل وفي بعض الأحيان مصحوب بفقدان اهداب الخلايا الظهارية وأحيانًا مصحوبًا بوذمة تحت الظهارة. في الحالات الأكثر خطورة ، كان هناك احتقان في الأوعية الدموية و ارتشاح خلايا هتروفيل مع تقشر الطبقات الظهارية . يؤدي التقشر في النهاية إلى تراكم الإفرازات في التجويف. في حالات نادرة كان هناك تقشر شديد يؤدي إلى فقدان الأجزاء العليا من الطبقة الظهارية. أظهت المقاطع النسجية في رئة الطيور المصابة تسلل الخلايا الالتهابية المكونة بشكل رئيسي من خلايا هتروفيل في النسيج الخلالي. في بعض العينات كان هناك نزيف واسع النطاق يملأ تجاويف الرئة وتسلل من الخلايا الحمضة و خلايا الهتروفيل مع نزيف خلالي. يمتد النزف أحيانًا إلى تجويف القصبة الهوائية. تتكون مجموعة الخلايا الالتهابية في بعض العينات بشكل أساسي من الضامة مع بعض الخلايا الليمفاوية. من هذه الدراسة خلصنا إلى أن الفيروسات المنتشرة في العراق تسبب المزيد من الآفات الرئوية عند مقارنة الآفات في القصبة الهوائية.

Introduction

Infectious bronchitis is an acute extremely infectious respiratory illness caused by the avian gamma-corona virus (1). Chickens and other avian species can be infected with infectious bronchitis virus (2). Infection with IBV predisposes the bird to subsequent bacterial infection, worsening the situation. Infection causes severe morbidity and variable mortality in broilers, as well as a significant decrease in layer production of eggs (3). The virus may be found all around the world and is spread by respiration or direct bird-to-bird contact or exposure to contaminated equipment, litter, tools, or more premises. Although in-ovo spread of the pathogen did not recorded yet, it may contaminate the eggshells by shedding from the reproductive or alimentary system (1,4). The virus is an enveloped virus that varies in morphology from round to pleomorphic. The virions are roughly 120 nm in size and have club-shaped outer appendages called spikes and these are approximately 20 nm long, giving the virus a look of a crown. Corona is a latin word means crown (5).

The symptoms of IB in affected young birds include general respiratory signs such as nasal secretion, respiratory rales, coughing, sneezing, and gasping (1). Watery eyes and even dilated sinuses have been seen in chicks (2). The chicks could be spotted curled up next to a heat source and seem depressed. Feed intake and growth might both be decreased. If the flock is not properly investigated, the sickness may even go undetected (1,2). Even in situations with evident production reductions and the laying of bleached eggs, respiratory abnormalities in laying chickens might be absent or extremely slight. The degree of the production reduction can range from minor to severe, depending on parameters such as the viral serotype and birds immunity, the lay phase during which the infection occurred, and concurrent infections (6). The trachea, nasal cavity, and sinuses of affected hens contain exudate. During the acute infection, the air sacs may be frothy, then turbid and have a yellow caseous discharge. Inflammation of lung tissues that surround big bronchi. Infections with strains of renal tropism can cause enlarged, faint kidneys due to the occupation of tubules with urate (7,8).

Materials and methods

Sampling

The tissue for molecular detection was including trachea, lung and kidney while for histopathological study it was including just lung and trachea. Small pieces about 5 mm thick were placed in disposable histopathology cassettes in 100 ml container filled with neutral buffered formalin. This fixative solution was prepared according to (9). It was prepared by adding 100ml of 37% formalin, 4 gm of sodium phosphate monobasic and 6.5 gm of sodium phosphate dibasic together with distilled water to a total of 1 liter. Before sealing the container a piece of cotton is added above the cassettes to force them to sink inside the fixative.

Molecular Detection

Polymerase chain reaction (PCR) was employed using primers designed by (10). These primers include the forward primer f-IBV-S1 5`- GTT TAC TAC TAC CAA AGT GCC TT -3` and the reverse primer 5`- GTG TAA ACA AGG TCA CCA TTT A -3`. Those oligonucleotides target the S1 gene and produce a 448bp PCR product.

Histopathology

Samples that tested positive for IBV by molecular technique were processed for histopathology while negative cases were discarded. The histopathological study was conducted as follows:

Tissue processing

This step was performed according to (11) as follows: After a minimum period of 72 hr in the fixative, the tissues were subjected to increasing concentrations of alcohols from 70% to 100% for 2 hr each. Tissues were soaked in two jars of xylene for 2 hr in each. The tissues were embedded in two jars of heated to 60°C paraffin wax for 2 hr each. The samples are then casted in paraffin as rectangular blocks in preparation for microtomy.

Microtomy

A rotary microtome was used to cut tissues into ribbons 5 micron thick and floated over a water bath. The slices of tissues were fixed on histological slides dried ready for staining (12).

Hematoxylins and eosin Staining

Staining dyes were prepared according to Bancroft and Layton (12). To dissolve the paraffin wax the slides were palced in two jars of xylene for 5 minutes each. The slides are then placed in decreasing concentrations of alcohols from 100% to 70% for 2 minutes each. Afterward, soaking in distilled water. This is followed by soaking in hematoxylins for 10 minutes then in eosin for 1 minutes.

Results and Discussion

Tracheal Sections

Histopathological sections from IBV positive birds showed variable lesions from farm to another. In some cases there was mild heterophilic infiltration and some times accompanied by deciliation and sometimes accompanied by subepithelial edema (Figure 1, A). In more severe cases there was congestion of blood vessels along heterophilic infiltration along with desquamation of the epithelial layers as those in (Figure 1, B). Desquamation eventually leads to accumulation of exudate in the lumen (Figure 1, C). In rare cases there was severe desquamation leading to sloughing of the upper parts of the epithelial layer (Figure 1, D).

It is important to mention that some samples from IBV positive farms showed normal tracheal tissues with little to no histopathological lesions. The lack of histopathological lesions could be due to the timing of sampling. In the early days of infection there was no lesion especially during the first 3 days post infection (13). Vaccination with homologous strain could result in lack of histopathological lesions after challenge (14).

The variation in the histopathological lesions is expected because the samples were collected from different farms with different farm management practices and different vaccination programs. Vaccine types and method of vaccination affect the outcome of the clinical challenge (15). Bad management could contribute to the severity of the lesions (1). Other concurrent disease could have been contributing to the severity of some cases (16–18).

Pulmonary Sections

Histopathological section in a lung of infected bird showed inflammatory cells infiltration consisting mainly of heterophils in the interstitial tissue. In some samples there was extensive hemorrhage filling the atrial lumen and infiltration of eosinophils and heterophils along with interstitial hemorrhage (Figure 2). Hemorrhage sometimes extends to the parabronchial lumen. Inflammatory cell population in some samples consisted mainly of macrophages with some lymphocytes. No case with positive IBV results were found normal in histopathological appearance.

These histopathological findings are inconsistent and non specific for IBV. This statement agrees with other studies conducted experimental IBV infection (13,17,19). Other experimental studies resulted with different histopathological finding including congestion, hemorrhage, edema, inflammatory cells infiltration consisting of heterophils and mononuclear cells (7,20,21).

Chickens with IBV infection have edematous tracheal mucosa. Within 18 hours of infection, there is deciliation, sloughing of epithelia, and modest infiltration of heterophils and lymphocytes. Regeneration of the epithelial lining takes 2 days, whereas cilia takes 7 to 8 days to repair. After 7 days, there is a huge influx of lymphoid cells into the lamina propria and germinal centers development. Within 24 hours after infection, there is edema and desquamation of airsacs. Later, there will be formation of lymphoid nodules and heterophilic infiltration (22). Histological alterations in the Harderian gland following IB vaccination and challenge include an influx of plasma cells, hyperemia, and significant lymphoid follicle development (23). Due to the high economic losses which is caused by this disease, this study was conducted to evaluate the pathological changes that occurs in natural infection with IBV.

Conclusions:

From this study, we concluded that viruses circulating in Iraq causes more pulmonary lesions when compared lesions in the trachea.

Acknowledgment

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Conflicts of interest

The authors declare no conflict of interest

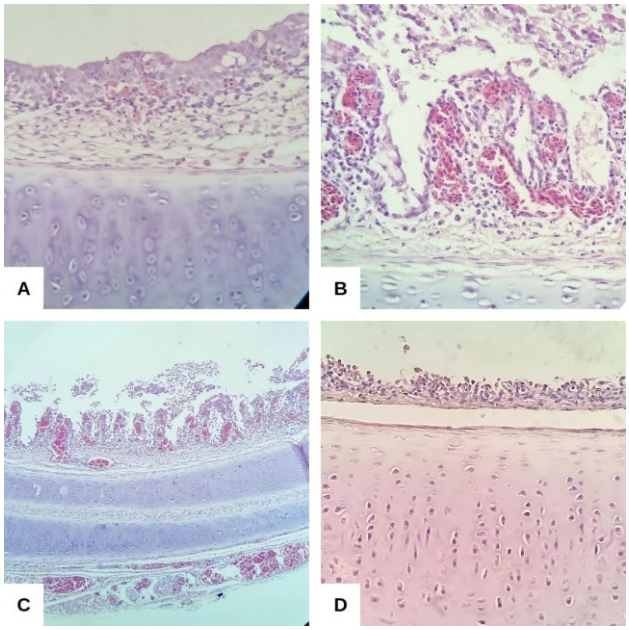

Figure 1: Histopathological sections in tracheas from IBV positive birds, H&E stain. A) Mild heterophilic infiltration accompanied by deciliation and subepithelial edema, 400X. B) Congestion of blood vessels along with heterophilic infiltration and desquamation of the epithelial layers, 400X. C) Congestion of blood vessels along with heterophilic infiltration and desquamation leading to accumulation of exudate in the lumen, 100X. D) Sloughing of the upper parts of the epithelial layer, 400X.

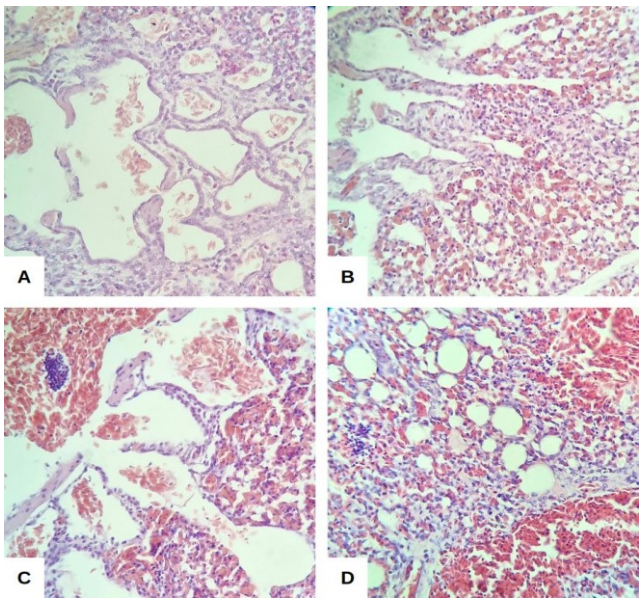

Figure 2: Histopathological sections in lungs from IBV positive birds, H&E stain 400X. A) Mild hemorrhage in the atrial lumen. B) Interstitial hemorrhage with inflammatory cell infiltration. C) Extensive hemorrhage in the atrial lumen with interstitial hemorrhage and inflammatory cell infiltration. D) Interstitial hemorrhage with inflammatory cell infiltration.

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